Journal of Surgical Oncology 1999;71:91–96

Irradiation-Induced Up-Regulation of Fas in Esophageal Squamous Cell Carcinoma Is Not Accompanied by Fas Ligand-Mediated Apoptosis

DAVID A. RIGBERG, MD, JOSEPH CENTENO, BS, FRANCES S. KIM, BS, BIBO KE, MD, KIM SWENSON, MD, MELINDA MAGGARD, MD, AND DAVID W. McFADDEN, MD, FACS*
Department of Surgery, UCLA School of Medicine, Los Angeles, California

Background and Objectives: Fas (APO-1) induces apoptosis after binding Fas ligand (FasL). Evidence suggests that tumors may use this interaction to evade the host immune response. Fas/FasL expression has not been reported in esophageal cancer. We hypothesized that Fas expression would render esophageal cancer cells susceptible to Fas ligation and that irradiation of the cells would increase Fas expression.
Methods: Two human esophageal squamous cell carcinoma lines, KYSE 150, which has a wild-type (wt) *p53* gene, and 410 (mutated *p53*), were irradiated. Reverse-transcriptase polymerase chain reaction was used to detect Fas and FasL expression. Fas protein was quantitated by enzyme-linked immunosorbent assay and its presence further confirmed by Western analysis. FasL was detected by Western analysis. Cells were treated with Fas monoclonal antibody (maximum 0.05 μg/ml) ± cycloheximide, and viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were also transduced with FasL cDNA and then quantified.
Results: Both lines expressed Fas and FasL, but only the KYSE 150 cell line displayed an increase in Fas following irradiation. No alteration in cell growth was detected for Fas antibody- or FasL-transduced groups versus controls.
Conclusions: We have demonstrated Fas and FasL expression in esophageal tumor lines. We have also shown that Fas levels are significantly increased in response to irradiation in a wt p53 line. However, cells were resistant to treatment with Fas antibody or following transduction with FasL, suggesting that these tumor cells may use Fas/FasL expression to evade the host immune response.
J. Surg. Oncol. 1999:71:91–96.

KEY WORDS: p53; Fas/CD95/APO-1; transduction

Presented at the 1998 American Gastroenterology Association Meeting, New Orleans, Louisiana.
Grant sponsor: Veterans Administration.
*Correspondence to: David W. McFadden MD, Division of General Surgery, UCLA School of Medicine, Los Angeles, CA 90095. Fax No.: (310) 825-6966. E-mail: p15057@mvs.medctr.ucla.edu
Accepted 9 March 1999

INTRODUCTION

Fas (CD95, APO-1) is a cell membrane protein that induces programmed cell death, or apoptosis, following ligation by its ligand, FasL [1]. A member of the nerve growth factor/tumor necrosis factor superfamily of receptors, Fas has been shown to be capable of inducing apoptosis subsequent to binding by either FasL or specific anti-Fas monoclonal antibodies [2,3]. Recent work suggests that Fas activates proteases in the interleukin (IL)-1β-converting enzyme (ICE) family via FADD/MORT (Fas-associated death domain protein) [4]. Previous studies have demonstrated the presence of Fas on a variety of normal cells and tumors, both hematopoietic and nonhematopoietic [5,6]. FasL is found predominantly on activated T cells and natural killer cells, although it has recently been described on the surface of tumor cells as well [7,8]. Thus, a role has emerged for the Fas/FasL interaction in which damaged cells expressing Fas undergo Fas ligation and apoptosis. Expression of FasL on the surface of tumor cells may play a role in tumor immunity as well [9].

Recent studies have described the upregulation of Fas following several treatments, including exposure to interferon gamma and tumor necrosis factor in B cells and gamma irradiation in breast and sarcoma lines [10]. These data have indicated a link between Fas upregulation and p53 mutational status in a number of cells, although wild-type p53 is not required for constitutive expression of Fas [11]. Associations have also been demonstrated between bcl-2 expression and the sensitivity of cells to treatment with FasL, further supporting a role for p53 in the Fas apoptotic mechanism [12].

Given that Fas can induce apoptosis and that it is present on tumor cells, novel therapies may be aimed at increasing its expression. Because radiation therapy is used extensively in the treatment of malignancies, it is of particular interest to determine the effects of radiation on Fas expression and its ability to induce apoptosis in these Fas-expressing cells.

Esophageal cancer continues to present a therapeutic dilemma, with complete surgical resection the only hope for cure [13]. Experiences with both chemotherapy and radiation have been disappointing, especially given the large number of cases that present at advanced stages [14]. We have characterized the response of human esophageal squamous cell carcinoma cells to ionizing radiation, with emphasis on p53 and its downstream genes [15]. In this study, we evaluate these lines for the constitutive expression of Fas and FasL as well as expression of Fas protein following irradiation. In addition, we examined the response of cells to Fas ligation via Fas monoclonal antibody and FasL transduction. Given the relationship to p53 reported in previous studies, we chose a line known to have a wild-type *p53* sequence and a line known to harbor a mutated *p53* gene. Characterization of the Fas/FasL interaction in these lines may provide the basis for future therapies of relatively resistant disease, especially considering that Fas/FasL expression by esophageal cancer has not been previously reported.

MATERIALS AND METHODS

Cell Culture

The esophageal squamous cell carcinoma lines KYSE 150 and 410 were a generous gift from Dr. Yutaka Shimada (Kyushu University, Kyoto, Japan). KYSE 150 has been shown by sequencing to have a wild-type *p53* gene, while KYSE 410 has a mutated gene [15,16]. Cells were maintained in a 1:1 combination of Hams F12 and RPMI-1640 media with 5% fetal bovine serum (GIBCO, Grand Island, NY). This was supplemented with 1% penicillin, streptomycin, fungizone solution (JRH Biosciences, Lenexa, KS), 25 μg/l gentamicin (GIBCO), 1% nonessential amino acid solution (GIBCO) and 1% sodium pyruvate (Sigma, St. Louis, MO). Conditions were controlled in a 5% CO_2 37°C incubator. Cell lines were detached with 0.25% trypsin (Clonetics, San Diego, CA) when confluent monolayers were present, approximately twice a week. Cells were pelleted for 10 min at 1,000 *g* in a refrigerated centrifuge at 4°C and washed with phosphate-buffered saline (PBS, Sigma). They were then respun at 1,000 *g* and resuspended in media following splitting. Cells used in experiments were passed fewer than 10 times; after 10 passages, frozen lines were thawed and used in their place.

Irradiation and Protein Collection

Cells were trypsinized, washed and reseeded into 60 mm tissue culture plates at 10^6 cells/plate (GIBCO) with 2 cc media. There were 18 plates for each cell line. These served in triplicate as control, 3 Gy and 6 Gy specimens at time points 6 and 24 hr. Plates were exposed to the appropriate gamma irradiation doses in a Mark I irradiator. Cells were exposed at a distance of 3″ to 7″ from the radioactive source and placed on a turntable during irradiation to ensure that all specimens received equal doses of radiation. Following treatment, cells were replaced into the incubator until ready for protein extraction.

Protein Extraction

Cells were washed twice with 37°C PBS following aspiration of the media and incubated with 1 ml of protein lysis buffer [PBS, triton X-100, sodium deoxycholate, and sodium dodecyl sulfate (SDS); Sigma] supplemented with 1.0 μg/ml pepstatin, 0.5 μg/ml leupeptin, 0.2 mM phenylmethyl sulfonyl fluoride (PMSF), and 1 mM ethylenediaminetetraacetic acid (EDTA) (all Sigma). Cell culture dishes were incubated at 4°C for 10 min and the cells removed by scraping with plastic spatulas (Fisher, Tustin, CA). Lysis buffer and cellular debris

were then transferred to microcentrifuge tubes and spun at 1,000 g for 10 min. Supernatant containing protein samples was decanted into fresh tubes and kept at –80°C until further use.

Standardization of Protein Specimens

Samples (10 μl) of each protein specimen were aliquoted into cuvettes along with 90 μl of the protein lysis buffer. Protein assay kit solutions were added (Bio-Rad, Hercules, CA), and specimens were allowed 15 min to develop as per the manufacturer's instructions. They were then read in a spectrophotometer at 750 nm. Bovine serum albumin (BSA) was used as a standard. Protein concentrations were then standardized to the specimen with the lowest content.

Determination of Fas Production

Standardized specimens were diluted 5:1 in sample diluent after pilot studies confirmed this as the optimal concentration for enzyme-linked immunosorbent assay (ELISA). The Fas content was determined using a Fas protein ELISA and the manufacturer's instructions (Calbiochem, Cambridge, MA). Fas protein concentration is determined in Fas units per milliliter via a standard curve constructed each time the ELISA kit is run. Results were read after development using an ELISA reader at dual wavelength of 450/550 nm. Separate specimens were used for verification of Fas protein via Western gel analysis. Briefly, 40 μg of protein were loaded on 10% SDS-polyacrylamide gel electrophoresis (PAGE) gels and run at 200 volts for 30 min. Proteins were then transferred onto a nitrocellulose membrane (Amersham, Arlington Heights, IL) for 2 hr. Membranes were blocked in 1% BSA for 2 hr at room temperature. We then probed the membranes with Fas primary antibody and used antimouse polyclonal antibody as a secondary (both Calbiochem). Finally, membranes were developed using a Western blot luminol reagent (Santa Cruz Biotechnology, Santa Cruz, CA).

Polymerase Chain Reaction (PCR) and Western Analysis for FasL

Cellular DNA was extracted from each of the lines using the QIAamp tissue extraction kit, following the protocol supplied (Qiagen, Chatsworth, CA). Primers were designed for FasL and PCR was performed in a 50 μl reaction containing 1 μg of genomic DNA. Primers (15 μM each) were used along with 45 μl of PCR supermix (GIBCO) and 10 μM of ^{32}PdCTP (Amersham). The reaction cycle consisted of 3 min of denaturization at 94°C, 32 cycles of 1 min at 94°C, 1 minat 59°C, and 1 min at 72°C. This was followed by 10 min of polymerization at 72°C. PCR products were run on a 1% agarose gel (Fisher) at 100 volts for 2 hr, and the gel was stained with ethidium bromide. Western gel was run using FasL monoclonal antibody as described above for Fas protein.

Antibody Treatment

Cells were grown in 96-well plates and exposed to increasing doses of Fas monoclonal antibody (maximum 0.05 μg/ml) ±cycloheximide for 6 to 48 hr (Sigma). Cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Acros, Beel, Belgium) assay. Following incubation, cells were washed and MTT (1.5 mg/ml) was added to the wells. Crystals were dissolved after 4 hr with dimethyl sulfoxide (DMSO, Sigma). Plates were then read on an ELISA reader at a wavelength of 450 nm. Relative survival of the cells was then calculated by comparing cell viability in the treated versus nontreated groups. The same protocol was also followed with the human T-cell line Jurkat E-6 (American Type Culture Collection, Rockville, MD), which is known to undergo apoptosis following treatment with Fas antibody [17]. These cells were cultured in the same medium as the KYSE lines. The contents of each well were aspirated and spun following wash steps to preserve the suspended cells. Viability was again assessed via MTT assay.

FasL Transduction

FasL cDNA in an E1-deleted adenovirus type 5 (AdV-5) was generously provided by the Dumont Transplantation Laboratory (UCLA, Los Angeles, CA). Their protocol for in vitro transduction with adenovirus FasL (AdV-FasL) was followed and is summarized here [18]. KYSE and Jurkat cells were plated on 100 mm culture dishes at 2×10^7 cells per plate. Medium was removed after overnight culturing, and 300 μl of either diluted virus stock (with or without FasL and both with β-galactosidase reporter gene) were added to each plate (in serum-free medium), leading to multiplicity of infection values of 10 to 20 plaque-forming units. Cells were incubated for 1 hr at 37°C, washed and then incubated in their standard media plus fetal bovine serum for 48 hr. At this time, gene transfer was assessed by direct histochemical staining for β-galactosidase protein, for which there was a reporter gene in the transduction vector. Briefly, cells were rinsed with PBS and then fixed on ice for 5 min in 2% formaldehyde plus 0.2% glutaraldehyde (both Fisher). Cells were again rinsed with PBS and then treated with 1 mg/ml x-gal reagent (GIBCO) in 5 mM 99% potassium ferricyanide (Sigma) and 2 mM $MgCl_2$ (Fisher) in PBS. Transduction was confirmed by direct observation of x-gal staining. In parallel experiments, cells were trypsinized following transduction and counted via a Coulter (Hialeah, FL) machine. Results were compared between control transduction and FasL transduction.

TABLE I. ELISA Results

Cell line	Control	3 Gy	6 Gy
KYSE 150	5.0 ± 0.14	8.8[a] ± 0.04	10.2[a] ± 0.14
KYSE 410	4.5 ± 0.01	4.6 ± 0.04	5.2 ± 0.13

Cell numbers as mean ± SEM.
[a]$P < 0.05$ vs. controls.

Statistical Analysis

ELISA results were tested by analysis of variance (ANOVA) to compare the mean values of the three separate experiments performed at each dose of radiation. Two replicants were used for each specimen. Statistical significance was at $P < 0.05$. Survival assays were evaluated by taking the means of similarly treated groups and verifying differences from controls by one-way ANOVA, with $P < 0.05$ representing significance.

RESULTS

Expression of Fas Protein

Both cell lines expressed Fas protein constitutively, and these levels were within 10% of each other, with KYSE 150 expressing 5.0 units/ml compared to KYSE 410 at 4.5 units/ml. Following irradiation with 3 Gy and 6 Gy, KYSE 150 was noted to increase Fas expression at the 6 hr time point with no further significant increase at 24 hr. There were no increases in Fas expression by KYSE 410 at 6 or 24 hr for the 3 Gy or 6 Gy dose. Results are summarized in Table I. Western gel analysis of protein extracts confirmed the presence of Fas in both cell lines as well (Fig. 1).

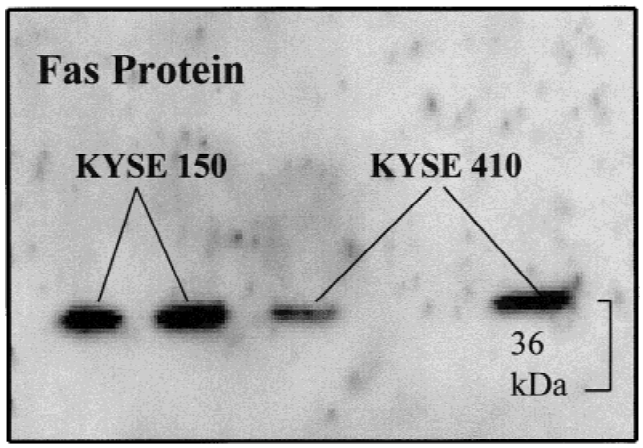

Fig. 1. Fas protein Western gel for KYSE 150 and KYSE 410. Both lines are shown here to produce the protein constitutively. KYSE 410 protein loaded at half strength (5 μl) in third lane and full strength (10 μl) in last lane.

Expression of FasL

Both KYSE 150 and KYSE 410 were found to express mRNA for FasL (Fig. 2). There were no dramatic differences in expression between radiated and control cells, although this was not a quantitative assay and only differentiation between expression and nonexpression would be expected. Western gel analysis confirmed the presence of FasL protein product as well (Fig. 3).

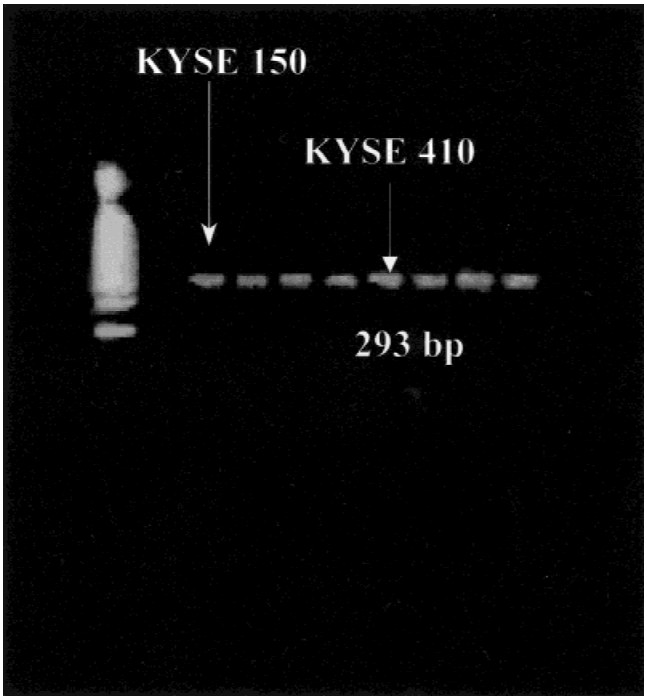

Fig. 2. FasL mRNA expression as shown by reverse-transcriptase polymerase chain reaction results. Lanes 1 and 2 show KYSE 150 controls; lanes 3 and 4 show irradiated KYSE 150 cells. Likewise, lanes 5 and 6 represent control KYSE 410 cells and lanes 7 and 8, irradiated cells. Both lines produced message at baseline and following irradiation.

Fas Antibody Treatment

Treatment with monoclonal Fas antibody did not lead to growth inhibition of either cell line. This included cells that were radiated at both the 3 Gy and 6 Gy doses to induce further Fas expression. In addition, following the failure of the Fas antibody to inhibit the growth of cells, we repeated the experiments in the presence of cycloheximide, which has been shown in previous studies to act synergistically with Fas antibody to induce apoptosis [2]. However, there were still no differences between cells treated with Fas antibody and controls. Treatment of Jurkat cells with Fas antibody resulted in significant decreases in cell numbers in a dose-dependent manner, with a maximum effect of 50% reduction in cell number (Fig. 4). Addition of cycloheximide did not have a significant effect on Jurkat cells.

FasL Transduction

As with treatment with Fas antibody, there were no apparent survival consequences following transduction

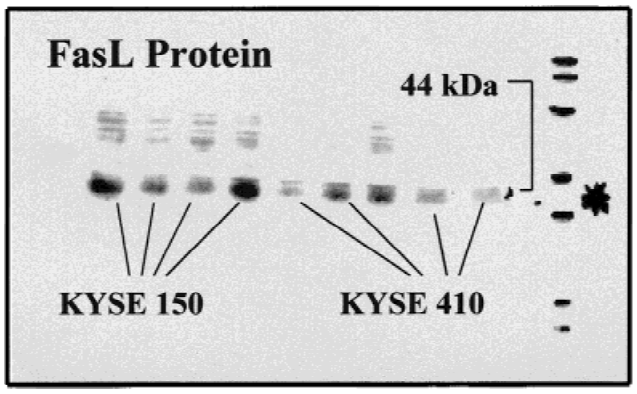

Fig. 3. Western gel for FasL protein displays bands for the 44 kDa product for both cell lines. **Lanes 1** and **2** were loaded with protein from control KYSE 150 cells (10 μl and 5μl), while **lanes 2** and **3** received protein from irradiated cells (5 μl and 10 μl). **Lanes 5** and **6** represent KYSE 410 controls (5 μl and 10 μl), while **lanes 7–9** were loaded with protein from irradiated cells (10 μl, 5 μl, 5 μl).

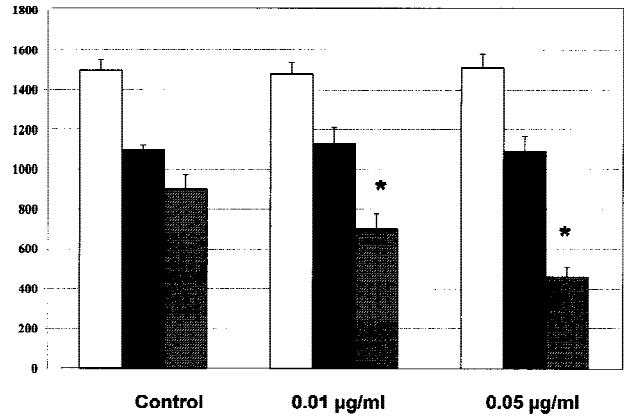

Fig. 4. Cell counts following treatment with increasing doses of Fas monoclonal antibody for KYSE 150 (open bars), KYSE 410 (solid bars) and Jurkat (hatched bars) cells. No effect was observed following treatment of either KYSE line, although significant decreases in Jurkat cells were observed. All data mean ± standard error. *$P < 0.05$ versus matched controls.

with FasL. cDNA transfer was assessed by direct histochemical staining for galactosidase protein with x-gal, which was positive in the FasL−transduced cells (40–50% transduction efficiency). The resultant cell numbers following transduction with FasL and control adenovirus are shown (Table II).

TABLE II. Fas Ligand Transduction

Cell Line	Control	FasL transduction
KYSE 150	3,516 ± 180	3,412 ± 214
KYSE 410	5,200 ± 241	5,314 ± 303
Jurkat	4,437 ± 312	3,009 ± 285[a]

Cell numbers as mean ± SEM.
[a]$P < 0.05$ vs. controls.

DISCUSSION

A number of studies have demonstrated that Fas is expressed by a variety of tumor types. Several studies have also demonstrated that it is possible to induce apoptosis in Fas-expressing cells by treating them with FasL or antibody [2,17]. While these findings suggest the possibility of treatment schemes targeted at Fas, they also suggest that methods to increase its expression may make cells more susceptible to Fas-mediated apoptosis. It was thus the aim of this study to not only characterize the expression of Fas in esophageal cancer cells, but also to seek to manipulate these levels with irradiation. Finally, we sought to determine whether these esophageal cancer cells would respond, as other studies have shown for different tumor types, to Fas ligation [2].

Two recent reports describe upregulation of Fas following treatment of cells in vitro. Sheard et al. [11] examined a series of tumors, including breast carcinoma, leiomyosarcoma, osteosarcoma, and fibrosarcoma lines; they found a dose-dependent increase in Fas expression starting at a dose of 2 Gy. Williams et al. [19] demonstrated upregulation of Fas in murine mastocytoma cells following treatment with cisplatin or etoposide; cells with upregulation of Fas were also found to be more sensitive to treatment with anti-Fas antibody.

Our results are in agreement with those of previous studies describing Fas upregulation following irradiation of tumor cells with wild-type *p53* genes. These increases occurred in a dose-dependent fashion and were statistically significant only for the wild-type *p53* expressing the KYSE 150 line. It should be noted that the KYSE 410 line does in fact increase expression of a mutated p53 protein following irradiation. However, it does so to a much lesser extent than its wild-type counterpart, and it is interesting to note the discrepancy in Fas upregulation between these two lines [15]. Sheard et al. [11] found a direct correlation between p53 status and upregulation of Fas following irradiation, and we demonstrated a trend toward increase in the mutated line we studied. As has been reported for other cells, constitutive expression of Fas is not dependent on p53 status.

Despite the presence and upregulation of Fas protein, neither of the esophageal cancer lines examined in this study had any response to treatment with anti-Fas monoclonal antibody or transduction with FasL. Both of these protocols have been previously described. Ligation of Fas by this particular antibody has been shown to induce apoptosis in several cell lines, and transduction with this adenoviral FasL successfully ligates Fas [2,18]. This resistance to treatment may be secondary to several factors, including the possibility of mutations in Fas. Furukawa et al. [20] have described a Fas mutation in the cytoplasmic "cell-killing" domain of the protein. Loss of function mutations in the Fas antigen have been seen in several autoimmune disorders, particularly forms of autoimmune

hepatitis, and have recently been demonstrated in a subset of multiple myeloma patients [21,22]. A further proposed mechanism for resistance to Fas-mediated apoptosis includes the presence of large amounts of soluble Fas protein that competitively binds to FasL [23]. Soluble Fas is a distinct form of the protein resulting from splicing that shortens the domain anchoring the protein to the cell membrane [24]. Recent sequencing of the FADD gene is also leading to investigation in mutations of this downstream Fas mediator [25].

This resistance to Fas-mediated apoptosis coupled with constitutive expression of FasL in these two cell lines suggests the possibility of a Fas counterattack model as a mechanism of immune escape in esophageal squamous cell carcinoma. It has been proposed that tumor cells may exploit this intrinsic cell death program on activated T lymphocytes. Apoptosis can be induced in tumor-infiltrating lymphocytes, which are inherently sensitive to Fas-mediated cell death [9]. The finding that these esophageal cells are themselves resistant to the Fas-mediated apoptotic pathway may render the counterattack model more effective.

In summary, our results show that the cell surface Fas receptor can be upregulated following gamma irradiation, suggesting that increases in Fas expression following irradiation may have relevant consequences in the responses of some cancer patients to radiation therapy. The fact that the wild-type p53 cell line upregulated Fas expression to a much greater extent than the small trend observed for the mutant KYSE 410 line coupled with previous reports suggests that p53 plays a role in increasing Fas expression. Further characterization of Fas expression in esophageal tumor lines may demonstrate more clearly whether this Fas upregulation is clearly linked to p53 status.

Despite the increased expression of Fas, neither cell line demonstrated sensitivity to anti-Fas monoclonal antibody or FasL transduction. Resistance to Fas-mediated apoptosis coupled with constitutive FasL expression suggests that these proteins may give certain tumors immune privilege. This may be particularly true for these relatively radio-resistant squamous cell tumors.

REFERENCES

1. Itoh N, Yonehara S, Ishii A, et al.: The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. Cell 1991;66:233–243.
2. Meterissian SH, Kontogiannea M, Po J, et al.: Apoptosis induced in human colorectal carcinoma by anti-fas antibody. Ann Surg Oncol 1997;4:169–175.
3. Suda T, Takahashi T, Golstein P, et al.: Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family. Cell 1993;75:1169–1178.
4. Chinnaiyan AM, O'Rourke K, Tewari M, et al.: FADD, a novel death-domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. Cell 1995;81:505–512.
5. Owen-Schaub L, Meterissian S, Ford RJ: Fas/APO-1 expression and function on malignant cells of hematologic and nonhematologic origin. J Immunother 1993;14:234–241.
6. Moller P, Koretz K, Leithauser F, et al.: Expression of APO-1 (CD95), a member of the NGF/TNF receptor superfamily, in normal and neoplastic colon epithelium. Int J Cancer 1994;57:371–377.
7. Gutierrez-Steil C, Wrone-Smith T, Sun X, et al.: Sunlight-induced basal cell carcinoma tumor cells and ultraviolet-B-irradiated psoriatic plaques express Fas ligand (CD95L). J Clin Invest 1998; 101:33–39.
8. Shiraki K, Tsuji N, Shioda T, et al.: Expression of Fas ligand in liver metastases of human colonic adenocarcinomas. Proc Natl Acad Sci USA 1997;94:6420–6425.
9. Walker PR, Saas P, Dietrich PY: Role of Fas ligand (CD95L) in immune escape: The tumor cell strikes back. J Immunol 1997; 158:4521–4524.
10. Nagata S, Golstein P: The Fas death factor. Science 1995;267: 1449–1456.
11. Sheard MA, Vojtesek B, Janakova L, et al.: Up-regulation of fas (CD95) in human $p53^{WILD\text{-}TYPE}$ cancer cells treated with ionizing radiation. Int J Cancer 1997;73:757–762.
12. Zhan Q, Alamo I, Yu K, et al.: The apoptosis-associated γ-ray response of $bcl\text{-}X_L$ depends on normal p53 function. Oncogene 1996;13:2287–2293.
13. Bhansali MS, Patil PK, Badwe RA, et al.: Historical control bias; adjuvant chemotherapy in esophageal cancer. Dis Esophagus 1997;10:51–54.
14. Stein H: Esophageal cancer: Screening and surveillance. Dis Esophagus 1996;9(Suppl 1):3–19.
15. Rigberg DA, Kim FS, Blinman TA, et al.: p21 expression is increased by irradiation in esophageal squamous cell carcinoma. J Surg Res 1998;76:137–142.
16. Tanaka H, Shibagaki I, Shimada Y, et al.: Characterization of p53 gene mutations in esophageal squamous cell carcinoma cell lines: Increased frequency and different spectrum of mutations from primary tumors. Int J Cancer 1996;65:372–376.
17. Owen-Schaub LB, Radinsky R, Kruzel E, et al.: Anti-Fas on nonhematopoietic tumors: Levels of Fas/APO-1 and bcl-2 are not predictive of biological responsiveness. Cancer Res 1994;54: 1580–1586.
18. Swenson KM, Ke B, Wang T, et al.: Fas ligand gene transfer to renal allografts in rats. Transplantation 1998;65:155–160.
19. Williams BA, Makrigiannis AP, Blay J, et al.: Treatment of the P815 murine mastocytoma with cisplatin or etoposide upregulates cell-surface Fas (CD95) expression and increases sensitivity to anti-Fas antibody-mediated cytotoxicity and to lysis by anti-CD3-activated killer-T cells. Int J Cancer 1997;73:416–423.
20. Furukawa F, Kanauchi H, Wakita H, et al.: Spontaneous autoimmune skin lesions of MRL/n mice: Autoimmune disease-prone genetic background in relation to Fas-defect MRL/1pr mice. J Invest Dermatol 1996;107:95–100.
21. Landowski TH, Qu N, Buyuksal I, et al.: Mutations in the Fas antigen in patients with multiple myeloma. Blood 1997;90:4266–4270.
22. Pensati L, Costanzo A, Ianni A, et al.: Fas/Apo1 mutations and autoimmune lymphoproliferative syndrome in a patient with type 2 autoimmune hepatitis. Gastroenterology 1997;113:1384–1389.
23. Suzuki N, Ichino M, Mihara S, et al.: Inhibition of Fas/Fas ligand-mediated apoptotic cell death of lymphocytes in vitro by circulating anti-Fas ligand autoantibodies in patients with systemic lupus erythematosus. Arthritis Rheum 1998;41:344–353.
24. Hughes DP, Crispe IN: A naturally occurring soluble isoform of murine Fas generated by alternative splicing. J Exp Med 1995; 182:1395–1401.
25. Kim PK, Dutra AS, Chandrasekharappa SC, et al.: Genomic structure and mapping of human FADD, an intracellular mediator of lymphocyte apoptosis. J Immunol 1996;157:5461–5466.